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Structure, Function and Inhibition of Poly(ADP-ribose)polymerase, member 14 (PARP14).

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Abstract: Poly(ADP-ribose)polymerase, member 14 (PARP14, alternatively named ARTD8, BAL2, and COAST6) is an intracellular mono(ADP-ribosyl) transferase. PARP14 transfers a negatively charged ADP-ribose unit from a donor NAD^+ molecule onto a target protein, post-translationally. PARP14's domain architecture consists of three macrodomains (Macro1, Macro2 and Macro3), a WWE domain and an ARTD (or catalytic domain). The Macro2 and Macro3 domains bind ADP-ribose (ADPr) with high affinity, whereas the WWE domain stabilizes the protein structure by binding to ADPr derivatives. The catalytic domain is involved in binding the NAD^+ and catalyzing the mono-ADP-ribosylation reaction. PARP14 has been identified as a possible anti-cancer and anti-inflammatory target. Acting as a transcriptional co-activator for STAT6, PARP14 acts to promote the over activation of the Th2 immune response, thus promoting the metabolic change to an anaerobic state (Warburg effect) and activation of cell survival pathways through JNK2 and the PGI/AMF complex. These changes are consistent with the metabolic sophistication observed in cancer, and the immune imbalance in inflammatory diseases. Current literature on selective and unselective PARP14 inhibitors are reviewed and discussed. Although there is no evidence that selective PARP inhibitors would be advantageous we have proposed some strategies for future design of selective PARP14 inhibitors.

Keywords: PARP14; Cancer; Inhibitors; ARTD8; BAL2; COAST6; MAR; ARTD

1. INTRODUCTION

1.1. The PARP superfamily

The Poly(ADP-ribose) polymerase (PARP) family consists of 18 genes which encode 17 intracellular ADP-ribose transferase enzymes (ARTDs) [1]. PARP enzymes post-translationally transfer negatively charged ADP-ribose groups from donor NAD^+ molecules onto their target proteins. The transfer reaction is facilitated by the highly conserved catalytic domain within all members of the superfamily [1]. PARPs contain a donor loop (D-loop) and acceptor loop in their catalytic site for stabilizing ligand bonds and strengthening ties with nearby PARPs [2]. Through poly(ADP-ribosyl)ation (PARylation) of the target protein PARPs control a wide array of biochemical processes. PARylation is especially prevalent during transcription regulation, stress responses, DNA repair, RNA interface, mitochondrial function, cell division and formation of sub-nuclear bodies [3].

The superfamily of PARP proteins was originally identified from the homology of PARP1, with most now having readily available crystal structures online through The Protein Data Bank (Table 1) [1, 4]. ARTDs nomenclature has been proposed for the PARPs since not all PARPs are enzymatically active and some function as mono(ADP-ribosyl) transferases (MARs) rather than Poly(ADP-ribose) polymerase (PARs) (Table 1) [5]. The PARP superfamily can be subcategorised into: DNA-dependent PARPs (PARP1, PARP2 and PARP3); tankyrases (PARP5A, and PARP5B); the CCCH (Cys-Cys-Cys-His) PARPs (PARP7, PARP12, and PARP13); and the macroPARPs (PARP9, PARP14 and PARP15). The other five PARPs (PARP4, PARP6, PARP8, PARP10, PARP11 and PARP16) have distinct domain structures and are not part of a subfamily [1, 3].

1.2 PARylation and MARylation

PARs of the PARP superfamily create polymers of branched or elongated (linear) ADP-ribose (ADPr) residues by their transfer reaction [6]. The polymerization event is promoted by the H-Y-E motif, located within the catalytic domain of PARP1-5a/b (PAR-generating) [7]. These members use the glutamate residue (E988) in their catalytic domain to assist with the transfer of numerous ribose units, and the eventual elongation of the target protein [7]. During PARylation of PARPs with a target protein, the PARP uses one NAD^+ as a donor ADPr unit to generate one ADPr and one nicotinamide. The cleaved ADPr is then attached to the Lys residue or the carboxylic acid of the Glu or Asp on the target protein [3]. The protein modifications are accelerated by the histidine and tyrosine of the motif, which guide NAD^+ into close proximity with the PARP catalytic domain [7]. The elongation reaction proceeds when the adenine-proximal ribose (A-ribose) units from the PAR chain termini are joined to an $\alpha(1\rightarrow2)$ O-glycosidic bond. The branching reaction occurs between two nicotinamide-proximal ribose (N-ribose) rings [3]. The specific structure of the PARs is thought to inhibit apoptotic pathways under stressful or hypoxic events [8]. The stress leads to synthesis of PAR-generating PARPs that contain

molecular material for the inhibition of mitogen activated protein kinases (MAPK), and further, the inhibition of apoptosis in dependent cells [8]. MAPK pathway inhibition is a target for cancer therapy in cancer cell lines that rely upon PARs function [8].

MARs are a subfamily of PARPs that share the highly conserved catalytic domain, however, lack the glutamate residue at position 988 [1]. In place of glutamate, MARs hold an isoleucine, leucine or valine. The subtle difference in the MARs subfamily does not allow the enzyme to form linear or branched chain polymers [1]. Instead, MARs are capable of MARylation [2]. MARs activity has been linked to the Unfolded Protein Response (UPR), and signal transduction [1]. UPR involves upregulation of MARs and is a protective measure of a cell under high cellular demand [1]. Exhaustive UPR is a hallmark of cancer, as cell apoptosis may be avoided [7]. However, the investigation into the structure and function relationship of MARs, PARP14 inclusive, has not been extensive [7].

Table 1: PARP superfamily members classifications and functions.

Subclass	PARP family member	Alternative names	Enzymatic activity	Key functional domains	Crystal Structure (.pdb)
DNA-dependent	PARP1	ARTD1	PAR	ARTD, WGR, Zinc Fingers and BRCT.	YES
	PARP2	ARTD2	PAR	ARTD and WGR	YES
	PARP3	ARTD3	PAR	ARTD and WGR	YES
CCCH PARP	PARP7	ARTD14, TIPARP, RM1	MAR	ARTD and Zinc-fingers and WWE	YES
	PARP12	ARTD12, ZC3HDC1	MAR	ARTD and Zinc-fingers and WWE	YES
	PARP13	ARTD13, ZC3HAV1, ZAP1	inactive	Zinc-fingers and WWE	YES
Tankyrase	PARP5A	ARTD5, tankyrase 1	PAR	ARTD and Ankyrin repeat	YES
	PARP5B	ARTD6, tankyrase 2, PARP6*	PAR	ARTD and Ankyrin repeat	YES
MacroPARP	PARP9	ARTD9, BAL1	inactive	Macrodomain	YES
	PARP14	ARTD8, BAL2, COAST6	MAR	ARTD, Macrodomain and WWE	YES
	PARP15	ARTD7, BAL3	MAR	ARTD and Macrodomain	YES
	PARP4	ARTD4, vPARP	PAR	ARTD and BRCT	NO
	PARP6*	ARTD17	MAR	ARTD	NO
	PARP8	ARTD16	MAR	ARTD	NO
	PARP10	ARTD10	MAR	ARTD	NO
	PARP11	ARTD11	MAR	WWE	NO
	PARP16	ARTD15	MAR	ARTD	YES

ADP-ribosyl transferase (ATRD); B-aggressive lymphoma protein (BAL); Collaborator of signal transducer and activator of transcription 6 (COAST6); vault PARP (vPARP); zinc-finger antiviral protein 1 (ZAP1); zinc-finger CCH-type antiviral protein 1 (ZC3HAV1); zinc-finger CCH domain-containing protein 1 (ZC3HDC1). Enzymatic activity: mono- (MAR), or poly- (PAR)[1, 3] *PARP6 which refers to two different proteins.

1.3. PARP14 structure

PARP14 contains three macrodomains (Macro1-3), a WWE domain and a catalytic domain, (Fig. (1)) [9, 10]. PARP14, like PARP10, contains RNA recognition motif (RRM) domains. The PARP14 Macrodomains are ADPr-binding modules that do not have allosteric contact with one another [9]. Each tightly packed domain is supported by a seven-stranded β-sheet with two or three α-helixes on each side. Macro1 binds ADPr with low affinity whereas Macro2 and Macro3 bind ADPr with high affinity. The Macro2 and Macro3 of PARP14 only interact with MARylated but not PARylated substrates, thus can distinguish between MAR and PAR as shown by the macrodomains detecting PARP10-dependant MARylated substrates both *in vitro* and in cells [9]. Therefore, not only is PARP14 able to act as a post-translational modifier through its catalytic domain, but also as a modification reader through its unique macrodomain.

Containing the conserved Trp (W) and Glu (E) residues, the WWE domain stabilizes the protein structure, and preferably binds to ADPr derivatives. A possible enzymatic target for the WWE domain has been suggested since mono ADPr binds preferentially over iso ADPr moieties [11].

The catalytic domain contains a NAD^+ binding pocket, or catalytic site, that is made up of a "base", "walls" and a "lid". The base is formed by β-sheet 3 and a loop connecting α-helix 3. The walls are formed by β-sheet 4 and α-helix 4. The lid is formed by a loop between α-helix 3 and β-sheet 4 (called the D-loop 1622-1626) [11]. Key binding residues in the catalytic site include: hydrogen bonding residue Asp1604; aromatic residues Tyr1633 and Tyr1646; along with other residues, such as His1601, Gly1602, Thr1603, Ser1607, Ser1641 and Thr1645. Outside of the catalytic site, Tyr 1620 is a key aromatic residue [11]. Another site that is located within the catalytic domain is the adenosine subsite. Key binding residues in the adenosine subsite include: hydrogen bonding residue Ser1722; Aromatic residues Tyr1727 and Tyr1714; along with other residues, such as Gly1683.

Fig. (1). Schematic representation of the PARP14 domain architecture *[9, 12]*.

2. PARP14

2.1. Known Function

PARP14 is the largest PARP enzyme, containing 1,801 amino acids, including those that form the characteristic macrodomain folds of the macroPARP subfamily [3]. The macrodomain folds are ADPr-binding modules that facilitate the localisation of macroPARPs for MARylation [3]. Homologous to the human BAL2b, a member of the B aggressive lymphoma family (BAL), PARP14 functions as a protective mediator in B cells. Expression of PARP14 enables the cell to avoid apoptosis and increase rate of B cell differentiation [13].

2.1.1 STAT6 Activation

As its alias Collaborator of signal transducer and activator of transcription 6 (CoaSt6) entails, PARP14 is crucial for the activation and activity of the signal transducer and activator of transcription factor 6 (STAT6), a protein which promotes the proliferation and differentiation of $CD4^+$ T-helper 2 (Th2) cells [14, 15]. The initial activation of STAT6 by the janus kinase mediated tyrosine phosphorylation (JAK/STAT) pathway, is promoted by PARP14 [16]. The phosphorylation process is initiated by the extracellular cytokine interleukin-4 (IL-4), which binds to the N-P-X-Y tyrosine residue on the JAK receptor along with an unphosphorylated STAT6 molecule. The binding of both substrates gives the activated complex, phosphorylating STAT6, which then dimerises and translocates to the nucleus [13, 16, 17]. However, this pathway is inhibited by the genes suppressor of cytokine signaling 1 (SOCS1) and SOCS2, which are stimulated in response to cytokines secreted by Th1 cells (IL-6, IL-10 and interferon-gamma). PARP14's MARylation of the SOCSs genes inactivates their expression, thereby ensuring the continual activation of STAT6 [14].

PARP14's involvement in the functional capacity of STAT6 occurs within the nucleus, and acts to provide STAT6 with access to the promoter region of the trans-acting T-cell-specific transcription factor (GATA3). In the absence of PARP14, histone deacetylase complex 2 (HDAC2) and HDAC3 bind to the promoter region of GATA3, preventing its expression [18]. PARP14 catalysed MARylation of these complexes causing HDAC2 and HDAC3 to disassociate from the gene, allowing STAT6 to bind to the palindromic 5'-TTC…GGA-3' repeat site, and activate transcription of the gene [15, 18, 19]. It is seen that in the absence of PARP14, STAT6 mediated signaling was notably impaired due to its interactions at both the activation and function of the transcription factor [14, 17, 18].

2.1.2 B Cell Differentiation

As a result of PARP14s involvement in STAT6 activation, and consequential expression of GATA3, it is evident that the enzyme plays a crucial role in Th2 differentiation [20-22]. Furthermore, using murine models, it is suggested that PARP14 promotes the upregulation of 2,314 B cell genes involved in glycolysis, and/or mitochondrial activity, thereby presenting PARP14 as a key mediator of B cell metabolic fitness, maturation and survival [14].

PARP14's influence on the upregulation of these metabolic and survival genes promotes the release of Th2 cell major cytokines, IL-4, IL-13 and IL-10, thereby promoting the humoral (type II) immune response. IL-4's endogenous effect on T-cells causes the differentiation into Th2 cells to be favored, via activation of the JAK/STAT pathway and amplification of its own expression [18]. IL-4 also activates the Akt signaling pathway, which involves the activation or inhibition of numerous genes, resulting in the enhancement of cell cycle progression, glucose metabolism, and cell growth, whilst suppressing apoptosis [16]. Furthermore, IL-4 activates

the immunoglobulin class switching of B cells towards the production of IgE and IgG1, promoting the inflammatory response [23]. Studies have noted that PARP14 is directly required to achieve maximum IL-4 induced B cell pro-survival signaling [18, 24]. Effects of activating the release of IL-13 are similar to that of IL-4, as both interleukins activate the IL-4 R alpha binding subunit. However, IL-13 also stimulates the genes for the mitogen-activated protein kinases ERK1 and ERK2, which promote cell survival, differentiation and metabolism [25].

2.1.3 JNK1/JNK2 Signaling Pathway

JNK signalling has a well-established role in apoptosis, cell proliferation, transformation and survival, via substrate phosphorylation [26]. The isoforms, JNK1 and JNK2 are ubiquitously expressed and considered redundant kinases [26, 27]. JNK2 is associated with various human cancers, often proving an essential enzyme in the growth of lung glioblastoma, skin and prostate cancer [26]. JNK2 regulates the cytoplasmic PARP14, which binds to and suppresses JNK1 activity when tested under physiological conditions [26]. Overexpression of PARP14 is noted to completely suppress JNK1 activity. Studies which deplete endogenous JNK2 display an enhanced rate of apoptosis in multiple myeloma cell lines, however, by inducing PARP14 expression, the enhanced apoptosis was completely reversed, and JNK1 expression restored [26]. Furthermore, through overexpression of PARP14 and the consequential suppression of JNK1, the M2 isoform of pyruvate kinase (PKM2) expression levels increase [27]. In its tetrameric form, PKM2 catalyses the rapid conversion of phosphoenolpyruvate (PEP) and ADP to pyruvate and ATP (Fig. (2)) [28]. Actively dividing cells (including cancers) predominantly express the PKM2 isoform in order to facilitate their high demand for ATP [28, 29]. The dimerized isomer of PKM2 acts as a nuclear kinase, translocating to the nucleus where it activiates a number of genes, including c-Myc and and hypoxia-inducible factor 1α [30]. Thus, PARP14 has a role in improving the glycolytic fitness of cancer cells. Both *in vitro* and *in vivo* analyses of c-Myc-driven burkitt lymphoma and multiple myelomas show that PARP14 expression is a critical mediator of cell survival [13, 16, 26, 31].

2.1.4 Interactions with PGI/AMF Complex

PARP14 MARylation of the protein autocrine motility factor/phosphoglucose isomerase (AMF/PGI) complex activates the dual functioning protein, causing upregulation of cellular energy production and survival [16]. Acting as an extracellular cytokine, AMF stimulates the Akt cell signaling pathway, whilst its role as the glycolic enzyme (PGI) converts glucose-6-phosphate to fructose-6-phosphate- a step preceding ATP generation, thereby promoting the energy production [15].

2.1.5 Homologous recombination

Like the DNA-dependent PARPs, PARP14 plays a role in DNA repair, by way of homologous recombination (HR). It has recently been suggested that PARP14 induced MARylation of the specialised DNA polymerases is required for HR, as both tissue and murine models demonstrate that in the absence of PARP14, HR can be initiated, but not completed [12]. It is suggested that PARP14 is involved in the strand invasion step, enabling the D-loop extension of the RAD51 foci by MARylating the RAD51 gene [12].

2.2. PARP14 as a potential anti-cancer target

Cancer cells require a large amount of energy to sustain their rapid growth, as well as cellular adaptations to prevent apoptotic mechanisms, which would prevent growth. This is partially facilitated by a sophistication in cellular metabolism from aerobic to anaerobic (regardless of oxygen availability), known as the Warburg effect [31]. Evidence suggests that the Warburg effect is the result of the over-activation of pro-survival signaling pathways, many of which, including HIF-1α and cMyc, are induced by PARP14 activation [32]. PARP14's interaction with promoting the growth of Th2 cells, inhibiting apoptotic pathways and increasing the inflammatory response can all be seen in association with promoting the progression of cancer. This has been demonstrated in hepatocellular carcinoma (HCC) cells; PARP14 levels are increased in cancer cells when compared to normal, healthy cells [27]. When the PARP14 protein was inhibited (using a shRNA knockdown model) the cancer cells had a significantly slower rate of growth while the normal cells growth rate was not affected, posing a PARP14 inhibitor as a method to selectively target cancer cells over normal cells [15].

2.3. PARP14 as a potential anti-inflammatory target

PARP14's role in promoting the differentiation of Th2 cells has sparked interest as to its association with the inflammatory response, and immune regulation [19]. Through transgenetic mice models, it has been observed that STAT6 overactivation, and consequential hyper-Th2 environment predisposes mice to develop allergic inflammation [33]. Many inflammatory disorders, including eosinophilic esophagitis (EoE), atopic dermatitis (AD), asthma-related phenotypes of airway hyper responsiveness (AHR) and food allergies are characterised by an overactivation of STAT6 and an increase in Th2 cytokines [34, 35]. Due to PARP14's role in promoting the activation of STAT6, it is seen that inhibition of PAPR14 in EoE models can reduce inflammation and restore lung function [17]. Studies have also noted a positive correlation between PARP14 and expression of eotaxin-3 (CCL26), an eosinophil chemoattractant responsible for the increased eosinophil infiltration observed in EoE, and likewise, inhibition of PARP14 decreased CCL26 expression [24]. Therefore, the critical role in which PARP14 plays in the STAT6 mediated activation of Th2, and release of Th2 cytokines, present an opportunity to inhibit PARP14 in order to decrease hyper inflammation in conditions such as EoE [33]. However, a 2015 study contradicts these claims, suggesting that

PARP14's involvement in hyper allergic diseases is in fact part of the immune defense system, and silencing of the PARP14 gene accelerates the progression of such states [36]. PARP14 has been noted to increase the M1 macrophage phenotype, and anti-inflammatory immune response that counters the M2 proinflammation [36]. Observations of allergic skin inflammation correlates silencing of the PARP14 gene with an acceleration of disease progression due to the decrease of B cells, as a result of the attenuation of PARP14's B cell survival signaling [36]. Other conditions such as acute arterial lesions and chronic atherosclerosis show similar disease-accelerating patterns [36]. Therefore, the question as to whether PARP14 promotes the development of hyperallergic diseases through its promotion of a Th2 dominant environment, or if the activation of haematopoietic cell survival pathways assists the immune system in fighting allergic diseases is up for debate, and successful synthesis of a selective PARP14 inhibitor could be key to answering some of these questions.

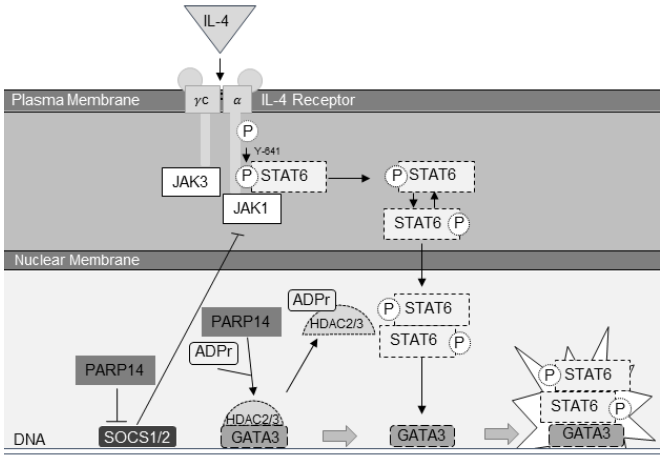

Fig. (2). Summary of PARP14's interactions with STAT6 induced activation of GATA3. PARP14 mediated MARylation of HDAC2 and HDAC3 cause the complexes to disassociate from the GATA3 promotor region allowing STAT6 to bind to the 5'-TCCNNNNGGA-3' repeat. PARP14 further inhibiting the expression of the suppressor of cytokine signaling genes (SOCS1 and SOCS2).

3.1 PARP Inhibitors

The first general PARP inhibitors (substituted benzamides) were identified in 1980. With the exception of coumarine and indole derivatives, which have been suggested to bind to one of the zinc finger domains, all known PARP inhibitors are nicotinamide mimetics that bind in the NAD^+ binding pocket of the catalytic domain as a competitive inhibitor [14]. PARP1 is the most active target of drug discovery and it is likely that these inhibitors are not selective to PARP1 over the other family members. The first PARP inhibitor Lynparza (also known as olaparib (AZD2281) KuDOS Pharmaceuticals and AstraZenca) was approved by many regulatory bodies worldwide in 2014 for the treatment of ovarian, fallopian tube and primary peritoneal cancer in women who have BRCA1 or BRCA2 mutations and have received three or more chemotherapy treatments. Lynparza is an oral PARP inhibitor with an IC_{50} of 2.9nM for PARP1 [37]. Lynparza also binds strongly to PARPs2-4 and weakly to PARP12, PARP15 and PARP16 [11]. Rucaparib (Clovis Oncolocy Inc.) is also an oral PARP1, PARP2 and PARP3 inhibitor being developed for advanced ovarian and metastatic castration-resistant prostate cancer.

3.2 Current status of PARP inhibitors and its shortcomings

Current PARP inhibitors, Lynparza and Rucaparib, work on the basis of synthetic lethality, targeting the DNA-repair PARPs which have a clearly established role in base excision repair and single stranded break repair. As the BRCA mutation prevents cells from completing HR, inhibition of the PARPs completely abolishes the cell's ability to repair DNA, thereby generating a lethality towards BRCA mutated cells over non-mutated cells [38]. They further act as chemosensitizers (reducing the apoptotic threshold) when combined with DNA damaging agents such as temozolomide or cisplatin [37]. The shortcomings of the current PARP inhibitors are that they only traget breast cancer patients with specific BRCA mutations. This makes their use limited to a small proportion of the population. There is a need for cancer treatments that can target a broader range of cancer types, for example, metastatic cancers. PARP14 has been shown to affect the ability for these cancer cells to grow and become metastatic, therefore targeting PARP14 may enable the treatment of a broader range of cancers.

3.3. Known inhibitors for PARP14

Recent knowledge about the structure and function of PARP14 has it identified as a possible anti-cancer and anti-inflammatory target. Although there is a great deal of structural similarity between the catalytic domain of different PARPs, there is scarce structural information on PARP14 inhibitors. PARP inhibitors to date are mostly non-selective inhibitors and tend to inhibit multiple PARPs. There is no evidence indicating whether selective or non-selective PARP inhibitors are more advantageous for treating disease, although highly selective PARP inhibitors have not yet been reported.

3.3.1 Unselective inhibitors

The first reported PARP14 inhibitors were published as part of a family-wide chemical profiling paper on PARPs [11]. This work identified inhibitors, computationally, as being either selective to PARPs1-4 or non-selective. Fig. (3) shows the non-selective PARP inhibitors that had favorable binding to PARP proteins, including PARP14. Investigation of compounds **1**, **2**, **3**, and **5** with PARP14 indicated key hydrophobic interactions with residues Tyr1633, Tyr1624, Tyr1640 and Tyr1646 and hydrogen bonding residues, Ser1641 and Gly1602 [11]. These non-selective inhibitors have no reported inhibition data with PARP14.

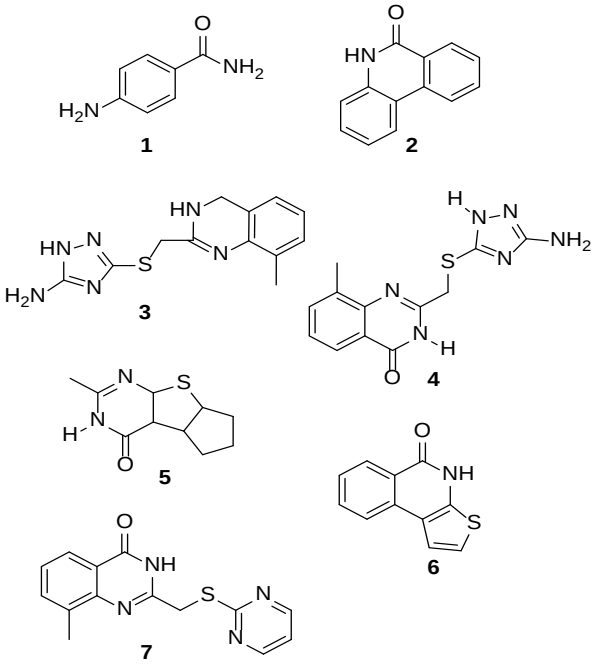

Fig. (3). Chemical structures of compounds **1** to **7** published with unselective PARP inhibition.

Crystal structures of **1** with PARP14 show a key hydrophobic interaction with Tyr1633 and hydrogen bonding interaction with Ser1641 (3GOY.pdb and 3SE2.pdb). Compound **2** with PARP14 also demonstrates key hydrophobic interactions with Tyr1624, Tyr1633, Tyr1640 and Tyr1646 and hydrogen bonding with Gly1602 and Ser1641 (3SE2.pdb). A crystal structure of **3** with PARP14 indicated a unique hydrophobic interaction at the opening of the pocket with Tyr1620. Other interactions include hydrophobic interactions with Tyr1620, Tyr1633, Tyr1646 and hydrogen bonding with Gly1602 and Ser1641 (3SMI.pdb). Another crystal structure from this set of compounds was compound **5** with PARP14. The key interactions include hydrophobic interactions with Tyr1633, Tyr1640 and Tyr1646 and hydrogen bonding with Gly1602 and Ser1641 (3SMJ.pdb) [11].

3.3.2 General PARP14 inhibitors

Benzothiazoles **8**-**11** are potent inhibitors of PARP14 showing μM inhibition (Fig. (4)). There is no information on selectivity or inhibition of other PARP enzymes with these compounds. The crystal structure of PARP14 with **11** shows key hydrophobic binding interactions with Tyr1714 and Tyr1727, and hydrogen bonding with Ser1722. No interactions were observed with Tyr1620 (4PY4.pdb) [39].

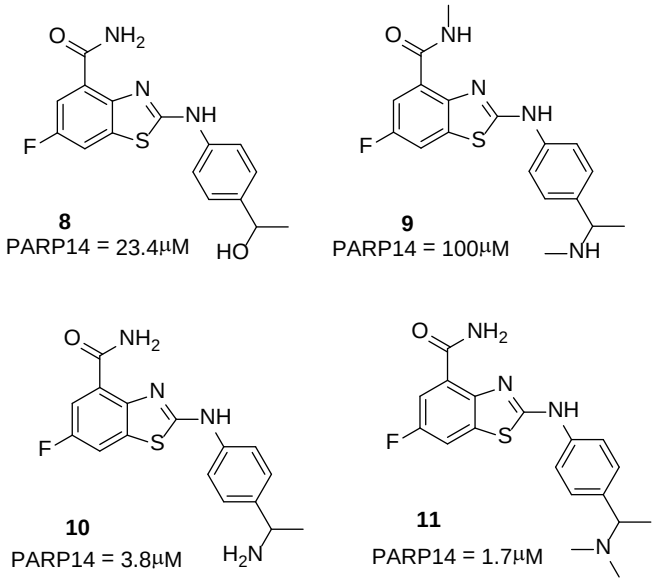

Fig. (4). Chemical structures of compounds **8** to **11** published with no selectivity information against PARP superfamily. IC_{50} values are shown next to the compounds.

3.3.3 Structural Activity Studies of PARP14 inhibitors

Work reporting PARP14 inhibitors as a virtual screening library identified two main inhibitors of PARP14, **12** and **13** (Fig. (5)) [19]. The second generation library, from this group, were synthesized and gave more structural insight into how to selectively target PARP1, PARP10, PARP14 and PARP15. This work demonstrated that placing the anilide moiety in para-position on the benzamide region, or alkylating the benzamide, proved detrimental to enzyme inhibition in all PARPs tested. Minor preference for PARP14 was shown when a methyl-ester group was present as in compound **25** (Fig. (5)) [40].

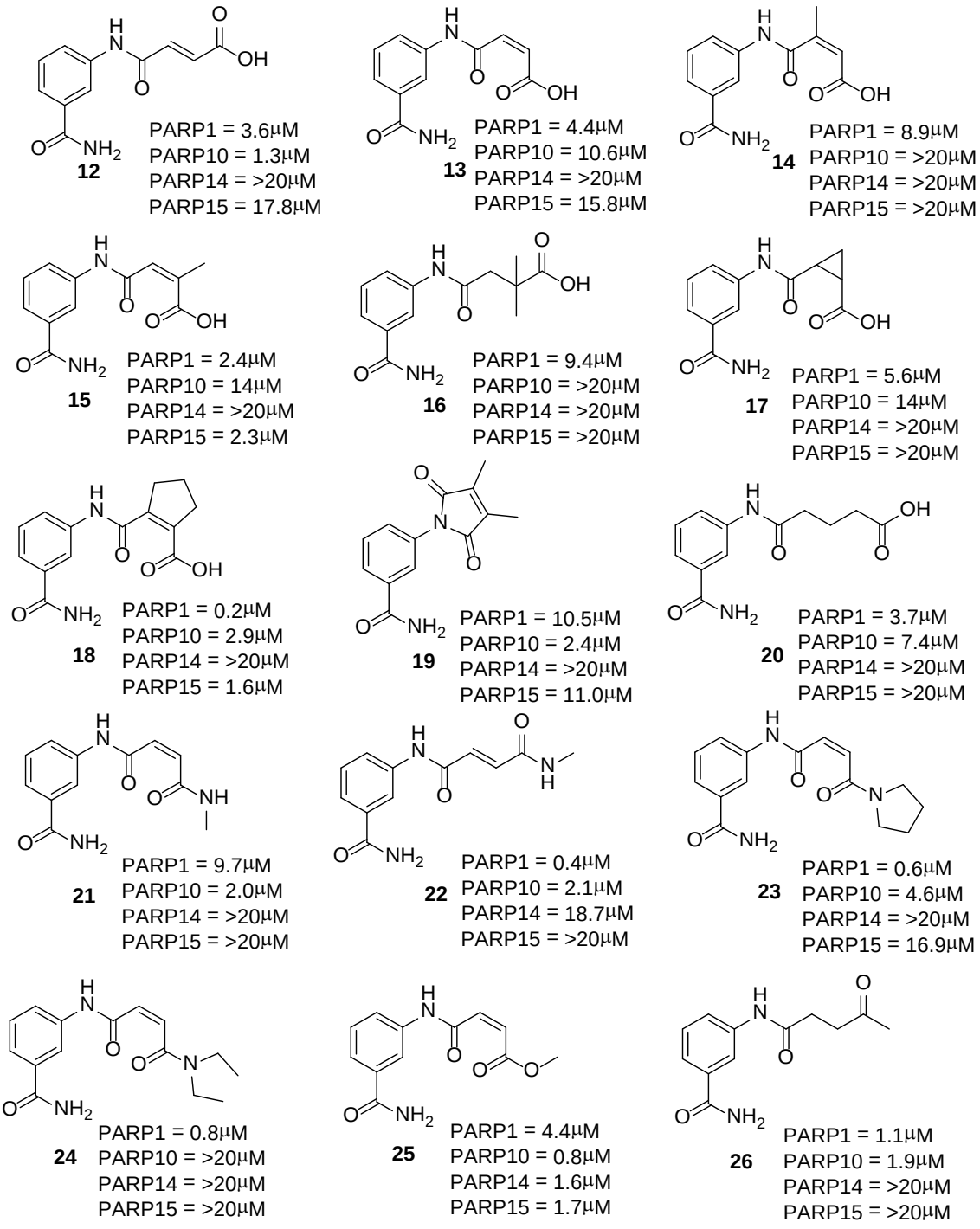

Fig. (5). Chemical structures of compounds **12** to **26** that search for selectivity against PARP superfamily. IC_{50} values are shown next to the compounds.

A crystal structure of compound **12** bound in the catalytic domain of PARP14 shows key binding interactions with Tyr1633, Tyr1640, Tyr1646 and hydrogen bonding with Gly1602 and Ser1641(4F1Q.pdb). Similarly, compound **13** showed hydrophobic interactions with Tyr1633, Tyr1640 and Tyr1646 and hydrogen bonding with Gly1602, Asn1624, Tyr1640 and Ser1641 (4F1L.pdb). Again no interactions were observed with Tyr1620 [19].

Peng *et al.* have published a high-throughput synthesis and screening of >1000 compounds using a small molecule microarray based strategy. The compounds were bidentate inhibitors which bound to both the primary pocket (catalytic site) and secondary pocket (adenosine binding subsite) in PARP14. This method identified **27** a potent PARP14 inhibitor that is 20 times more selective for PARP14 over PARP1 (Fig. (6)) [41]. Two other potent inhibitors were identified, **28** and **29**, which were less selective over the other PARP enzymes. A crystal structure of **27** bound in the catalytic domain of PARP14 shows key binding interactions with Thr1713 and Phe1697 and hydrogen bonding with Thr1684. Secondary binding was observed between the benzamide group and adenosine subsite residues Tyr1727, Tyr1714 and Tyr1721 and hydrogen bonding with Asn1698 (5LYH.pdb). A crystal structure of **28** showed similar interactions with the benzamide group and adenosine subsite residues Tyr1721, Tyr1727, Tyr1721 and Tyr1714 as well as hydrogen bonding with Gly1683 and Ser1722. The other end of **28** interacted with Tyr1726 (5LXP.pdb).

Yoneyama-Hirozane *et al.* have identified PARP14 inhibitors using a novel auto-ribosylation method. They screened a diverse library of approximately 500,000 small compounds from a library at Takede Pharmaceutical Company. Two compounds **30** and **31** were identified as potent inhibitors with a high degree of selectivity over PARP1 (Fig. (6)). The crystal structures of these two compounds both show interactions with the adenosine subsite residues Tyr1727 and hydrogen bonding between Ser1722 and Gly1683 (5V7T.pdb and 5V7W.pdb). Compounds **31** also has additional interactions with Tyr1714 (5V7W.pdb) [42]. Neither of these compounds bound to the catalytic site only the adenosine subsite.

Upton *et al.* have also targeted both the catalytic site and adenosine binding subsite in PARP14 hoping to achieve selectivity for PARP14 over the other PARP enzymes. They have produced 3 generations of inhibitors, each generation giving further SAR information and increasing in potency. Generation 3 piperazinyl-amide derivatives **32**-**37** display poor selectivity for PARP14 over PARP1 and PARP5a but are highly potent with **32** being the most potent PARP14 inhibitor to date (Fig. (6)).

Compounds **33-35** all displayed potency below 1 μM (Fig. (6)). Activity was lost when a heteroaryl group was added to the piperazine ring as shown with **36**. Also, addition of another aromatic ring as in compound **37** decreased the effectiveness of the inhibitor [43]. A crystal structure of **35** shows the aminobenzamide moiety bound into the nicotinamide pocket with interactions between the fluorinated aryl ring and Leu1755, Val1773, Val1784 and Leu1782 (5NEQ.pdb).

3.3. Possible Future Strategies for Designing Selective PARP14 Inhibitors

Comparison of the catalytic site of PARP14 with the other PARPs (PARPs1-4, PARP5a, PARP5b, PARPs9-10, PARPs12-16) showed that the "base" of the pocket is highly conserved [11]. Greater sequence variability is shown on the outer edges of the catalytic site, in particular, the D-loop [11]. The D-loop of PARP14 has a great deal of plasticity when compared to the longer more rigid D-loops in PARP1 and PARP2. This variability of the D-loops between the different PARPs could lead us to developing selective PARP inhibitors. PARP14 also presents a Tyr1620 residue in the D-loop region that is unique and not observed in other PARPs that were investigated [11]. Although this research did not investigate PARP7, PARP6, PARP8 and PARP10 due to the unavailability of crystal structures, it is clear that targeting the catalytic site may prove difficult. Within the catalytic domain there is a secondary binding site known as the adenosine subsite. When NAD^+ (nicotinamide adenine dinucleotide) binds to the catalytic domain the nicotinamide moiety binds into the catalytic site and the adenine moiety binds into the adenosine subsite. The adenine subsite is less conserved across the PARP superfamily members and compounds that are large and can bind to both of these sites within the catalytic domain may lead to designing selective PARP14 inhibitors [11].

Another possible way to selectively inhibit PARP14 over the other PARPs is to target the macrodomains. The macroPARPs (PARP9, PARP14 and PARP15) all contain macrodomains with PARP14 and PARP15 displaying mono(ADP-ribosyl) transferases (MARs) activity while PARP9 is inactive. PARP15 has two macrodomains and PARP14 has three macrodomains. The two PARPs have 72% identity when comparing the homology of their PARP catalytic sites which can be related to their evolutionary relationship [44]. Comparison of the PARP14 Macro1, Macro2 and Macro3 domains with PARP15 Macro1 and Macro2 domains show between 24.4% and 60.8% identity, with the most identity between PARP14 Macro3 and PARP15 Macro2. PARP14 Macro 3 has been shown to bind strongly to ADPr [44]. Homology modeling shows that ADPr based inhibitors may possibly target the macrodomains.

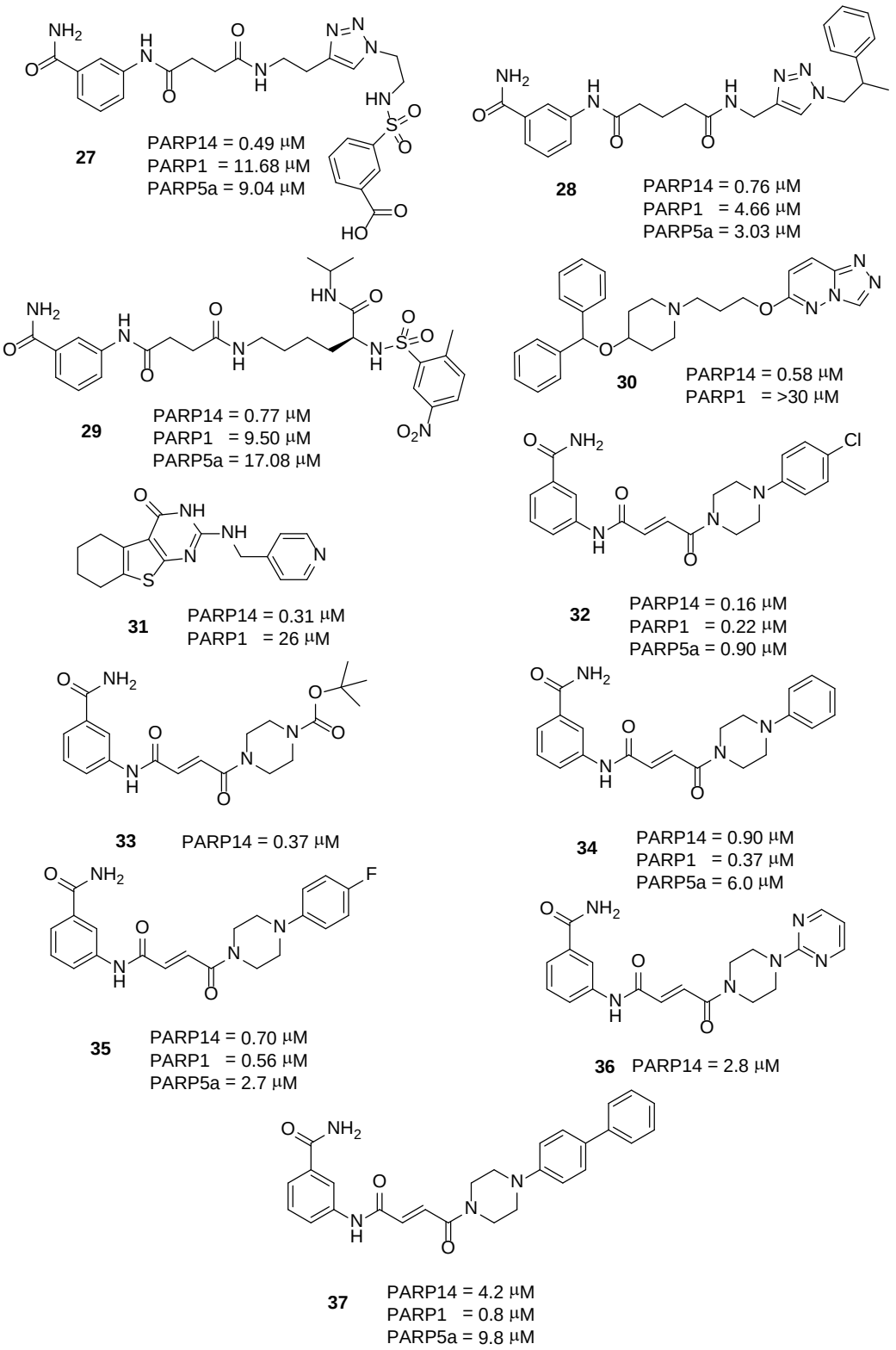

Fig. (6). Chemical structures of compounds **27** to **31** that search for selectivity against PARP superfamily. IC_{50} values are shown next to the compounds.

The possibility of targeting the WWE domain is another option with WWE domains being present in PARP7, PARP11, PARP12, PARP13 and PARP14. The WWE domains of PARP11 and PARP14 have been shown to bind ADPr derivatives including, ATP and ADPr for PARP11, and ADPr only for PARP14 [45, 46]. Although there is no information on inhibition of the PARPs upon binding of these compounds, it can be concluded that ADPr based inhibitors may also bind well to the WWE domain.

5 CONCLUSION

PARP14 has been identified as a promising anti-cancer and anti-inflammatory target. Acting as a transcriptional co-activator for STAT6, PARP14 acts to promote the over activation of the Th2 immune response, thus promoting a metabolic change to an anaerobic state (Warburg effect) and activation of cell survival pathways through JNK2 and the PGI/AMF complex. These changes are consistent with the metabolic sophistication observed in cancer, and the immune imbalance in inflammatory diseases. Thus, designing PARP14 inhibitors represents a new area of research, with PARP14 recently identified as a possible anti-cancer and anti-inflammatory target. Current literature on PARP14 inhibition shows that designing a selective inhibitor could be possible, although there is yet no solid evidence that selective PARP inhibitors would be advantageous for treating any diseased state. The high conservation of the catalytic domain makes the possibility of designing inhibitors that are selective for PARP14 over the other 17 PARP family members challenging, unless targeting a unique residue within this domain that is outside of the catalytic binding site. We suggest that targeting the macrodomain and WWE domain may provide another option for producing selective inhibitors. One possible scaffold for targeting these domains could be derivatives of ADPr, representing a novel option for future design.

LIST OF ABBREVIATIONS

AD	Atopic dermatitis
ADPr	ADP-ribose
AHR	Airway hyper responsiveness
Akt	Protein Kinase B
A-ribose	Adenine-proximal ribose
AMF/PGI	Autocrine motility factor/phosphoglucose isomerase
ARTD	ADP-ribosyl transferase
BRCA1	Breast Cancer 1
BRCA2	Breast Cancer 2
BAL	B-aggressive lymphoma protein
COAST6	Collaborator of signal transducer and activator of transcription 6
EoE	Eosinophilic esophagitis
ERK1	Mitogen-activated protein kinase 1
ERK2	Mitogen-activated protein kinase 2
HCC	Hepatocellular carcinoma
HDAC2	Histone deacetylase complex 2
HDAC3	Histone deacetylase complex 3
HR	Homologous recombination
IL-4	Cytokine interleukin-4
JNK1	Kinase Jun N-terminal kinase 1
JAK/STAT	Janus kinase mediated tyrosine phosphorylation pathway
MAPK	Mitogen activated protein kinases
MAR	Mono(ADP-ribosyl) transferase
MARylation	Mono(ADP-ribosyl)ation
N-ribose	Nicotinamide-proximal ribose
PARP14	Poly(ADP-ribose)polymerase, member 14
PAR	Poly (ADP- ribosyl) transferase
PARylation	Poly(ADP-ribosyl)ation
PEP	Phosphoenolpyruvate
PKM2	Pyruvate kinase isoform M2
SOCS1	Suppressor of cytokine signaling 1
SOCS2	Suppressor of cytokine signaling 2
Th2	T-helper 2
UPR	Unfolded Protein Response
vPARP	Vault PARP
ZAP1	Zinc-finger antiviral protein 1
ZC3HAV1	Zinc-finger CCH-type antiviral protein 1
ZC3HDC1	Zinc-finger CCH domain-containing protein 1

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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